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(71) Applicant: THE GEORGE WASHINGTON UNIVERSITY MEDICAL CENTER [US/US]; 2300 Eye Street, N.W., Washington, DC 20037 (US).

(72) Inventors: GOLDSTEIN, Allan, L.; 6407 Bradley Boulevard, Bethesda, MD 20814 (US). FAGARASAN, Mirela, O.; Apartment 608 North, 950 25th Street, Washington, DC 20037 (US).

(74) Agents: REPPER, George, R. et al.; Rothwell, Figg, Ernst & Kurz, 555 13th Street, N.W., #701 East, Washington, DC 20004 (US).

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(54) Title: METHOD OF TREATING SEPTIC SHOCK USING THYMOSIN β_4

(57) Abstract

A method of treating septic shock in a mammal including the administration of a septic shock-treating effective amount of Tβ_4 to the mammal.

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METHOD OF TREATING SEPTIC SHOCK USING THYMOSIN β_4

The present invention relates to a method of treating septic shock in mammals.

Description of Background Art

Thymosin β_4 ("Tβ_4") is a peptide which has been reported as containing 43 amino acids. Amino acid sequence information on Tβ_4 is disclosed in U.S. Patent No. 4,297,276, herein incorporated by reference.

Tβ_4 has been found to be present in numerous tissue types in mammals and has also been implicated in a wide variety of cellular and physiological processes including inducing terminal deoxynucleotidyl transferase activity of bone marrow cells, stimulating secretion of hypothalamic luteinizing hormone releasing hormone and luteinizing hormone, inhibiting migration and enhancing antigen presentation of macrophages, and inducing phenotypic changes in T-cell lines in vitro.

Septic shock is a condition in which infection is widely disseminated in many areas of the body, the infection generally being disseminated through the blood from one tissue to another and causing extensive damage. Septic shock can occur with numerous medical conditions, including (1) peritonitis caused by the spread of infection from the uterus and fallopian tubes; (2) peritonitis resulting from rupture of the gut, sometimes caused by intestinal disease or wounds; (3) generalized infection resulting from spread of a

simple infection; (4) generalized gangrenous infection resulting specifically from gas gangrene bacilli; and (5) infection spreading into the blood from the kidney or urinary tract. Septic shock is of critical concern from a clinical viewpoint because, among other reasons, this condition frequently leads to death.

Although septic shock is a somewhat common clinical phenomenon, the mechanisms involved as well as the pathological changes remain poorly understood. For example, despite the treatment of bacterial infection, many patients deteriorate further, which may be due to clinical sequelae of hypotension with low systemic vascular resistance, renal insufficiency, adult respiratory distress syndrome, severe coagulopathy and severe metabolic dysfunctions. Thus, there is an urgent need in the art for effective methods of treating septic shock.

Summary of the Invention

In accordance with the present invention, a method of treating septic shock in mammals includes administering a septic shock-treating effective amount of $T\beta_4$ to said mammals.

Description of the Preferred Embodiments

The terms "Thymosin β_4" and "$T\beta_4$" refer to peptides having the amino acid sequence disclosed in U.S. Patent No. 4,297,276, supra.

Mammalian septic shock occurs in association with a series of events in the mammal's body referred to as the "sepsis cascade". The sepsis cascade typically begins with bacterial infection of the mammalian host resulting in release of bacterial toxins, introduction of endotoxin, activiation of host defense systems, i.e., plasma protein systems as well as cellular defense systems including endothelial cells,

macrophages, monocytes and neutrophils, with release of proinflammatory mediators including cytokines, lipid metobolites, proteases, toxic oxygen products, nitric oxide and adhesion proteins.

According to one aspect of the present invention, effective amounts of $T\beta_4$ are administered to a subject to reduce blood free radical levels in the subject, and thereby treat or prevent septic shock in the subject or obstruct progression of sepsis cascade in the subject. $T\beta_4$ has been found to reduce blood free radical levels almost as much as SOD (super oxide dismutase), an enzyme that eliminates free radicals. When a septic shock-treating or a septic shock-preventing effective amount of $T\beta_4$ is administered to a mammal, the blood levels of pathological mediators of bacteria-induced lethality are decreased in the mammal.

$T\beta_4$ has been found to obstruct the sepsis cascade in mammals. During sepsis, peroxidation of lipids in blood is increased (mMol of malonyldialdehyde), but returned to normal or about normal with $T\beta_4$ administration. Sepsis also reduces circulating blood leves of glutathione. However, administration of $T\beta_4$ returns circulatory gluthathine leves to normal or about normal.

As noted above, administration of $T\beta_4$ during sepsis decreases blood hydroperoxide levels and blood glutathione levels. Administration of $T\beta_4$ during sepsis also decreases cerebellar cGMP levels, and decreases the blood levels of arachidonic acid metabolites such as $Tx\beta_2$ and 6-keto-$PGF_1\alpha$, PAF, and cytokines such as IL-1α and TNF-α.

While not wishing to be bound to any particular theory, it is believed that reducing blood levels of pathological mediators of bacteria-induced lethality in

a mammal decreases the amount of infection in the mammal which, in turn, aids in obstructing the spesis cascade, and in preventing and treating septic shock.

Thus, according to the present invention, methods of treating and preventing septic shock in mammals are provided. The methods of the present invention include administration of septic shock-treating effective amounts, septic shock-preventing effective amounts and sepsis cascade progression-obstructing effective amounts of $T\beta_4$ to mammals.

According to preferred embodiments of the present invention, effective amounts of $T\beta_4$ are administered to subjects to treat or prevent septic shock in the subjects, or obstruct progression of sepsis cascade in the subjects. In these embodiments, the subjects preferably are human.

According to preferred embodiments of the present invention, compositions containing $T\beta_4$ may be formulated in a conventional manner for administration by any suitable route. Suitable routes of administration include, but are not limited to, oral, rectal, nasal, topical, vaginal, and parenteral (including subcutaneous, intramuscular, intravenous and intradermal). Particularly preferred embodiments utilize oral or parenteral administration, with parenteral administration being a more preferred embodiment. It will be appreciated that the preferred route may vary with the condition, age and species of the recipient.

While not essential, in preferred embodiments, $T\beta_4$ is administered as part of a pharmaceutical formulation. The formulations of the present invention comprise $T\beta_4$ together with one or more pharmaceutically acceptable carriers and optionally with other

therapeutic ingredients. The carrier(s) are "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form, e.g., tablets and sustained release capsules, and may be prepared by any suitable pharmaceutical methods.

Such methods include, but are not limited to, the step of bringing into association $T\beta_4$ with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association $T\beta_4$ with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of $T\beta_4$, as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion, etc.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine $T\beta_4$ in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered

compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations suitable for topical administration include lozenges comprising $T\beta_4$ in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising $T\beta_4$ in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising $T\beta_4$ to be administered in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising $T\beta_4$ and a pharmaceutically acceptable carrier, or may utilize a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration wherein the carrier is a solid include a coarse powder having a particle size, for example, in the range from about 20 to about 500 microns which is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations suitable for vaginal administration may be presented as tampons, creams, gels, pastes, foams or spray formulations containing, in addition to $T\beta_4$, suitable carriers.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may optionally contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other suitable agents having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

A proposed dose for administration of the compositions in the present invention is a septic shock-treating, septic shock-preventing or sepsis cascade progression-obstructing effective amount of $T\beta_4$, which can be in a range of from about 0.4 to about 4 mg of $T\beta_4$ per kg of body weight of recipient (mg/kg), preferably from about 1 to about 4 mg/kg. A dose can be administered to the patient daily, one or more times per day of administration, e.g., two or three times per day, and doses can be administered one or more days per week, e.g., two, three, four, five, six or seven days per week.

The invention is applicable to native (i.e., naturally occurring) $T\beta_4$ as well as synthetic $T\beta_4$ and recombinant $T\beta_4$ having the amino acid sequence of native $T\beta_4$, biologically active amino acid sequences substantially similar thereto, or a biologically active abbreviated sequence form thereof, and their biologically active analogs (including muteins) having substituted, deleted, elongated, replaced, or otherwise modified sequences which possess bioactivity substantially similar to that of native $T\beta_4$.

In accordance with one embodiment of the present invention, $T\beta_4$ can be administered in combination with a therapeutically effective amount of another substance useful in treating septic shock such as, for example, antibiotics, or antibodies (polyclonal or monoclonal) directed to antigens located on endotoxins. Of course, the acceptable dosage range of the other substance will depend upon its properties (i.e., the acceptable dosage range will depend upon what other substance is being administered).

$T\beta_4$ and another substance useful in treating septic shock can be administered "in combination" which, as defined herein, includes various schemes designed to administer $T\beta_4$ and the other substance to a subject, whether or not the other substance and $T\beta_4$ are administered separately or together, such that the desired dosages of $T\beta_4$ and the other substance are present in the subject at the same time. Any suitable scheme can be used to administer $T\beta_4$ and another substance useful in treating septic shock "in combination" in accordance with the present invention.

Suitable dosages of either $T\beta_4$ alone or $T\beta_4$ in combination with another substance useful in treating septic shock may be administered 1 to 6 times or more

per day. The precise dose administered will depend on the age, condition and other factors of the recipient.

The following examples are for illustrative purposes only, and are not to be construed in a limiting sense.

Example 1

Synthetic $T\beta_4$ was provided by Alpha 1 Biomedicals, Inc. (Two Democracy Center, 6903 Rockledge Drive, Ste. 1200, Bethesda, Maryland 20817). $T\beta_4$ was prepared by solid phase peptide synthesis.

Swiss-Webster mice 4-6 weeks of age (20 - 25g) were housed 6 per cage. The mice were divided into 2 groups: endotoxic mice (endotoxin 60 mg/kg i.p. in acute treatment) and endotoxic mice treated with $T\beta_4$, 5 minutes and 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 hours post administration of the endotoxin. Survival was recorded 2 times a day for 7 days. The results are presented in Table I below. As can be seen, $T\beta_4$ protected against lethal endotoxin shock and increased the survival rate to as much as 100%.

Example 2

Swiss-Webster mice 4-6 weeks of age (20-25g) were divided into groups as follows.

1. Mice treated with a lethal dose of endotoxin (60 mg/kg).
2. Mice treated with 60 mg/kg endotoxin followed by an injection of 100 μg $T\beta_4$ (5 minutes post administration of endotoxin).
3. Mice treated with 60 mg/kg endotoxin followed by 2 injections of 100 $\mu g/T\beta_4$ (immediately following and 2 hours post administration of endotoxin).
4. Mice treated with 60 mg/kg endotoxin followed by 3 injections of 100 μg $T\beta_4$ (immediately following, 2 and 4 hours post administration of endotoxin).

The results presented in Tables I and II below indicate that $T\beta_4$, administered 3 times post administration of endotoxin, increased the survival rate of mice treated with endotoxin to 100%.

TABLE I

Experimental Groups	Survival of Swiss Webster Mice Following Lethal Endotoxin Dose and $T\beta_4$ (No. mice alive)			
	0 hrs	24 hrs	48hrs	72hrs
Endotoxin 60 mg/kg	7	1	1	1
Endotoxin 60 mg/kg + $T\beta_4$ 100 μg 1x	7	4	3	3
Endotoxin 60 mg/kg + $T\beta_4$ 100 μg 2x	7	4	4	4
Endotoxin 60 mg/kg + $T\beta_4$ 100 μg 3x	7	7	7	7

TABLE II

Protective Effect of $T\beta_4$ on Survival of Mice Treated with Lethal Doses of Endotoxin

	Number of Mice Alive					
EXPERIMENTAL GROUPS	0 hr.	24 hr.	48 hr.	72 hr.	7 day	14 day
Endotoxin 60 mg/kg	8	0				
Endotoxin 60 mg/kg +$T\beta_4$ 100 μg 3x	8	8	8	8	8	8

The results presented in Tables III - V below show effects of $T\beta_4$ respectively on mice cerebellar cGMP levels, blood serum TNF-α levels, and blood serum TNF-α and IL-1α levels.

TABLE III

$T\beta_4$ effects on cGMP cerebellar levels in mice treated with lethal doses of endotoxin

EXPERIMENTAL GROUPS	Cerebellar cortex cyclic GMP (pmol per mg/protein)
Control	10.1 ± 0.2
Endotoxin 60 mg/kg i.p.	30.7 ± 4.1
Endotoxin 60 mg/kg i.p.+$T\beta_4$ 0.8 mg/kg i.p.	12.1 ± 0.9

TABLE IV

Serum TNF-α levels in mice treated with lethal doses of endotoxin

EXPERIMENTAL GROUPS	TNF-α (pg/ml)		
	1 hr	3 hr	5 hr
Endotoxin 60 mg/kg	1098 ± 106	783 ± 38	623 ± 51
Endotoxin 60 mg/kg + $T\beta_4$ 100 μg	823 ± 79	535 ± 47	480 ± 45

TABLE V

Serum TNF-α and IL-1α levels* in mice protected against endotoxin-induced lethality by pretreatment with SOD and $T\beta_4$

Pretreatment	TNF-α (pg/ml)	IL-1 α (pg/ml)
None	4462 ± 123	1137 ± 123
SOD (3.3×10^4 μ/kg)	63 ± 5.1	53 ± 3.1
$T\beta_4$ 100 μg/mice	321 ± 26.2	298 ± 20

* Mice were pretreated with SOD (super oxide dismatase), 30 minutes before endotoxin administration XX blood for determined of TNFα and IL-1α levels was collected 1 hr after endotoxin administration.

Example 3

Using the same materials and methods as in examples 1 and 2, the time and dose-dependency of the protective effect of $T\beta_4$ on endotoxin lethality were studied. The results are presented in Tables II and III below.

As can be seen in Tables VI and VII, $T\beta_4$ had a protective effect on endotoxin toxicity when given immediately following, 2 and 4 hours after endotoxin treatment. The most effective protective dose was 100 μg $T\beta_4$ administered three times. Also, $T\beta_4$ partially increased the survival of mice treated with endotoxin when it was administered three times in doses of 50 μg and 20 μg $T\beta_4$. 10 μg $T\beta_4$ has also sometimes shown positive activity.

TABLE VI

Experimental Groups	Survival of Swiss Webster Mice Following Lethal Endotoxin Dose and $T\beta_4$ (No. mice alive)					
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Endotoxin 60 mg/kg	8	4	4	4	4	4
Endotoxin 60 mg/kg + $T\beta_4$ 100 μg, 3x; $T\beta_4$ was administered immediately following, 2 and 4 hrs. after endotoxin.	8	8	8	8	8	8
Endotoxin 60 mg/kg + $T\beta_4$ 100 μg, 3x; $T\beta_4$ was administered 2, 4 and 6 hrs. after endotoxin.	8	7	4	4	4	4
Endotoxin 60 mg/kg + $T\beta_4$ 10 μg, 3x; $T\beta_4$ was administered immediately following, 2 and 4 hrs. after endotoxin.	8	8	6	6	6	6

TABLE VII

Experimental Groups	Survival of Swiss Webster Mice Following Lethal Endotoxin Dose and $T\beta_4$ (No. mice alive)						
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs	5 days	6 days
Endotoxin 60 mg/kg	10	4	3	3	3	3	3
Endotoxin 60 mg/kg + $T\beta_4$ 50 μg, 3x; i.p. $T\beta_4$ was administered immediately following, 2 and 4 hrs. after endotoxin.	10	9	7	7	7	7	7
Endotoxin 60 mg/kg + $T\beta_4$ 20 μg, 3x; i.p. $T\beta_4$ was administered immediately following, 2 and 4 hrs. after endotoxin.	10	5	4	4	3	3	3
Endotoxin 60 mg/kg + $T\beta_4$ 10 μg, 3x; i.p. $T\beta_4$ was administered immediately following, 2 and 4 hrs. after endotoxin.	10	3	3	3	3	3	3

Example 4

Using the same methods and materials as in examples 1-3, the effect which $T\beta_4$ has on blood levels of IL-1α, TNF-α, PAG, $Tx\beta_2$ and 6-keto-$PGF_1\alpha$, which are pathological mediators of endotoxin induced lethality, was studied. The results are presented in Tables IV-IX below.

As can be seen in Tables VIII-XIII, $T\beta_4$ decreased IL-1α, TNF-α serum levels as well as PAF, $Tx\beta_2$ and 6-keto-$PGF_1\alpha$ plasma levels after administration of a lethal dose of endotoxin.

TABLE VIII

EXPERIMENTAL GROUPS	IL-1α pg/ml (serum levels)	
	1 hr	3hr
Endotoxin 60 mg/kg	492 ± 45, 2	550 ± 37, 1
Endotoxin 60 mg/kg + $T\beta_4$ 100 μg administered simultaneously	147,5 ± 15,1	207, 5 ± 19,3

TABLE IX

EXPERIMENTAL GROUPS	PAF (pg/ml) (plasma levels)			
	0.5 hr.	1 hr	2 hr	3 hr
Endotoxin 60 mg/kg	78 ± 6	279 ± 17	127 ± 13	55 ± 3
Endotoxin 60 mg/kg + $T\beta_4$ 100 μg administered simultaneously	63 ± 7	161 ± 17	66 ± 5	46 ± 5

TABLE X

EXPERIMENTAL GROUPS	$Tx\beta_2$ (pg/ml) (plasma levels)			
	0.5 hr.	1 hr	2 hr	3 hr
Endotoxin 60 mg/kg	1442 ± 103	2937 ± 258	912 ± 105	695 ± 65
Endotoxin 60 mg/kg + $T\beta_4$ 100 μg administered simultaneously	209 ± 9,5	607 ± 53	196 ± 18	112 ± 9

TABLE XI

EXPERIMENTAL GROUPS	6-keto-PGF1α (pg/ml) (plasma levels)			
	0.5 hr.	1 hr	2 hr	3 hr
Endotoxin 60 mg/kg	341 ± 31	1141 ± 112	897 ± 75	811 ± 7
Endotoxin 60 mg/kg + $T\beta_4$ 100 μg administered simultaneously	141 ± 9	261 ± 23	147 ± 19	121 ± 13

TABLE XII

EXPERIMENTAL GROUPS	PAF (pg/ml) (serum levels)		
	1 hr	3 hr	5 hr
Endotoxin 60 mg/kg	938	662	567
Endotoxin 60 mg/kg + $T\beta_4$ 100 μg administered simultaneously	807	591	496

TABLE XIII

EXPERIMENTAL GROUPS	TNF-α (pg/ml) (serum level)		
	1 hr	3 hr	5 hr
Endotoxin 60 mg/kg	938	662	567
Endotoxin 60 mg/kg + Tβ_4 100 μg administered immediately after endotoxin	807	591	496

While the invention has been described and illustrated with details and references to certain preferred embodiments, those skilled in the art will appreciate that various modifications, changes, omissions, and substitutes can be made without departing from the spirit of the invention.

Example 5

Using a septic shock model in rats (Sprague-Dawley, male, 200-225 g each), the effect of thymosin β_4 alone and together with antibiotics was studied. Peritonitis was induced in rats in the following way. A 1-cm incision was made into the peritoneal which exposed the cecum. A tight ligature was placed around the cecum with 4-0 suture distal to the insertion of the small bowel, forming an area of devitalized tissue while maintaining bowel continuity. A puncture wound was made with 16-gauge needle into the anti-mesenteric surface of the cecum and a small amount of fecal contents was expressed through the wound. The cecum was replaced into the peritoneal cavity, and the anterior peritoneal wall and skin were closed with

surgical staples. Each animal was given a bolus of normal saline (15 ml/kg) for hydration and allowed to recover overnight.

The results presented in Table XIX show Tβ_4 increased the survival of rats.

TABLE XIX

Survival of Sprague-Dawley rats following septic shock, antibiotics, and thymosin β_4

EXPERIMENTAL GROUPS	0 hr	24 hr	48 hr	72 hr
Rats with Septic Shock + antibiotic*	10	1	1	0
Rats with Septic Shock + antibiotic* + thymosin β_4 1 mg/rat x 3**	10	4	3	3

* gentamicin sulfate, 1 mg/rat
** At 0 hr, 2 hr, and 4 hr post induction of peritonitis

The results presented in Tables XX to XXIII below show effects of Tβ_4 respectively on rat cytokine levels, blood malonyldialdehyde levels, glutathione levels and arachidonic acid metabolic levels.

TABLE XX

The effect of Tβ_4 on Cytokine Levels in a septic shock model in rat

EXPERIMENTAL GROUPS	TNFα (pg/ml)	IL$_1\alpha$ (pg/ml)
Control	undetectable	undetectable
Rats with Septic Shock	2658 ± 197	1387 ± 123
Rats with Septic Shock + Tβ_4 1 mg/rat x 3 as above	1259 ± 138	853 ± 62

TABLE XXI

The effect of $T\beta_4$ on lipid peroxidation in a septic shock model in rat

EXPERIMENTAL GROUPS	Blood Levels of malonyldialdehyde (nNol MDA)	
Control	15.3 ± 1.3	18.6 ± 1.1
Rats with Septic Shock	49.1 ± 3.5	46.3 ± 3.8
Rats with Septic + $T\beta_4$ 1mg/rat x 3 as above	19.6 ± 1.7	16.8 ± 1.5

TABLE XXII

The effect of $T\beta_4$ on glutathione levels in a rat septic shock model

EXPERIMENTAL GROUPS	RBC GSH (μmoles/ml cells)	
Control	1.38 ± 0.1	1.46 ± 0.13
Rats with Septic Shock	0.43 ± 0.05	0.29 ± 0.04
Rats with Septic Shock + $T\beta_1$ mg/rat x 3 as above	1.45 ± 0.11	1.39 ± 0.12

TABLE XXIII

The effect of $T\beta_4$ on arachidonic acid metabolite levels in a septic shock model in rat

EXPERIMENTAL GROUPS	6-keto-PGF$_1$ (pg/ml)	T x β_2 (pg/ml)
Control	58 ± 3.5	89 ± 7.6
Rats with Septic Shock	1828 ± 145.3	3622 ± 295
Rats with Septic Shock + $T\beta_4$ 1 mg/rat x 3 as above	829 ± 59	1627 ± 123

The results presented in Tables XXIV and XXV below demonstrate that $T\beta_4$ increases the survival rate of rats with septic shock.

TABLE XXIV

EXPERIMENTAL GROUPS	Number of Rats Alive			
	0 h	24 h	48 h	72 h
Rats with Septic Shock	10	2	2	0
Rats with Septic Shock + $T\beta_4$ 1 mg/rat x 3 as above	10	3	3	3

TABLE XXV

EXPERIMENTAL GROUPS	Number of Rats Alive			
	0 h	24 h	48 h	72 h
Rats with Septic Shock	10	1	0	0
Rats with Septic Shock + $T\beta_4$ 1 mg/rat x 3 as above	10	4	3	3

While the invention has been described and illustrated with details and references to certain preferred embodiments, those skilled in the art will appreciate that various modifications, changes, omissions, and substitutes can be made without departing from the spirit of the invention.

What is claimed is:

1. A method of obstructing progression of a sepsis cascade in a mammal in which a sepsis cascade is occurring, comprising administering to said mammal a sepsis cascade progression-obstructing amount of thymosin β_4 ($T\beta_4$).

2. The method of claim 1, wherein said mammal is human.

3. The method of claim 2, wherein the $T\beta_4$ is administered at a dosage from about 0.4 to about 4 mg per kg of body weight of said mammal.

4. The method of claim 2, wherein the $T\beta_4$ is administered at a dosage from about 1 to about 4 mg per kg of body weight of said mammal.

5. The method of claim 3, wherein the $T\beta_4$ is administered parenterally.

6. The method of claim 5, wherein the $T\beta_4$ is administered intravenously.

7. The method of claim 1, wherein the $T\beta_4$ is synthetic $T\alpha_1$.

8. The method of claim 1, wherein said amount of $T\beta_4$ is sufficient to reduce in said mammal at least one member selected from the group consisting of cerebellar cGMP levels, blood malonyldialdehyde levels, blood glutathione levels, blood PAF levels, blood $Tx\beta_2$ levels, blood 6-keto-$PGF_1\alpha$ levels, blood IL-1α levels and blood TNFα levels.

9. A method of preventing septic shock from occurring in a mammal following endotoxin introduction in said mammal, comprising administering to said mammal a septic shock-preventing effective amount of $T\beta_4$, wherein septic shock is prevented in said mammal for at least 72 hours after administration of said $T\beta_4$.

10. The method of claim 9 wherein septic shock is prevented in said mammal for at least 120 hours after administration of said $T\beta_4$.

11. The method of claim 9 wherein septic shock is prevented in said mammal for at least 14 days after administration of said $T\beta_4$.

12. The method of claim 9 wherein said mammal is human.

13. The method of claim 12, wherein the $T\beta_4$ is administered at a dosage from about 0.4 to about 4 mg per kg of body weight of said mammal.

14. The method of claim 12, wherein the $T\beta_4$ is administered at a dosage from about 1 to about 4 mg per kg of body weight of said mammal.

15. The method of claim 13, wherein the $T\beta_4$ is administered parenterally.

16. The method of claim 15, wherein the $T\beta_4$ is administered intravenously.

17. The method of claim 9, wherein the $T\beta_4$ is synthetic $T\beta_4$.

18. The method of claim 9, wherein said amount of $T\beta_4$ is sufficient to reduce in said mammal at least one member selected from the group consisting of cerebellar cGMP levels, blood malonyldialdehyde levels, blood glutathione levels, blood PAF levels, blood $Tx\beta_2$ levels, blood 6-keto-$PGF_1\alpha$ levels, blood IL-1α levels and blood TNFα levels.

19. A method of treating septic shock in a mammal which comprises administering to said mammal a septic shock-treating effective amount of $T\beta_4$.

20. The method of claim 19, wherein said mammal is human.

21. The method of claim 20, wherein the $T\beta_4$ is administered at a dosage from about 0.4 to about 4 mg per kg of body weight of said mammal.

22. The method of claim 20, wherein the $T\beta_4$ is administered at a dosage from about 1 to about 4 mg per kg of body weight of said mammal.

23. The method of claim 22, wherein the $T\beta_4$ is administered parenterally.

24. The method of claim 23, wherein the $T\beta_4$ is administered intravenously.

25. The method of claim 19, wherein the $T\beta_4$ is synthetic $T\beta_4$.

26. The method of claim 19, wherein said amount of $T\beta_4$ is sufficient to reduce in said mammal at least one member selected from the group consisting of cerebellar cGMP levels, blood malonyldialdehyde levels, blood glutathione levels, blood PAF levels, blood $Tx\beta_2$ levels, blood 6-keto-$PGF_1\alpha$ levels, blood IL-1α levels and blood TNFα levels.

27. A pharmaceutical dosage unit containing an amount of $T\beta_4$ selected from the group consisting of a mammalian sepsis cascade progression-obstructing effective amount of $T\beta_4$, a mammalian septic shock-preventing effective amount of $T\beta_4$ and a mammalian septic shock-treating effective amount of $T\beta_4$, wherein said $T\beta_4$ is provided in a carrier which is pharmaceutically acceptable for administration to a mammal.

28. The pharmaceutical dosage unit of claim 27, containing from about 0.4 to about 4 mg $T\beta_4$ per kg of mammalian body weight.

29. The pharmaceutical dosage unit of claim 27, containing from about 1 to about 4 mg $T\beta_4$ per kg of mammalian body weight.

30. The pharmaceutical dosage unit of claim 28 which is in a form for parenteral administration, wherein the carrier is a sterile liquid carrier suitable for parenteral administration.

31. The pharmaceutical dosage unit of claim 30 which is in a form for intravenous administration.

32. The pharmaceutical dosage unit of claim 31, wherein the $T\beta_4$ is dissolved in a sterile isotonic saline solution.

33. The pharmaceutical dosage unit of claim 27, wherein said $T\beta_4$ is present in said pharmaceutical dosage unit in an amount sufficient to reduce in said mammal at least one member selected from the group consisting of cerebellar cGMP levels, blood malonyldialdehyde levels, blood glutathione levels, blood PAF levels, blood $Tx\beta_2$ levels, blood 6-keto-$PGF_1\alpha$ levels, blood IL-1α levels and blood TNFα levels.

34. The method of claim 1, wherein said administration of $T\beta_4$ reduces blood free radical levels in said mammal.

35. The method of claim 9, wherein said administration of $T\beta_4$ reduces blood free radical levels in said mammal.

36. The method of claim 19, wherein said administration of $T\beta_4$ reduces blood free radical levels in said mammal.

INTERNATIONAL SEARCH REPORT

Int. onal Application No
PCT/US 94/10879

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CANCER IMMUNOLOGY IMMUNOTHERAPY, vol.14, no.3, March 1983, BERLIN pages 145 - 150 ISHITSUKA H. ET AL. 'Protective Activity of Thymosin Against Opportunistic Infections in Animal Models' see the whole document ---	1-36
A	US,A,4 297 276 (GOLDSTEIN A.L.) 27 October 1981 cited in the application see the whole document -----	1-36

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 January 1995	25. 01. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

rnational application No.

PCT/US94/10879

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 1 to 26 and 34 to 36 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int onal Application No
PCT/US 94/10879

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4297276	27-10-81	NONE	